

## Rigid Isolation between the Northern Population and the Southern Population of the Medaka, *Oryzias latipes*

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**ABSTRACT** — The genetic characters of the wild populations of the medaka, *Oryzias latipes*, were studied in the boundary between the Northern Population and the Southern Population in Japan. The two major populations were found to be isolated from each other rigidly, and the boundary was well correlated with mountain barrier. The populations with a genotype different from both major populations were found in the quite limited area around the western end of the boundary.

### INTRODUCTION

In the course of the allozyme studies of *Oryzias latipes*, it has been shown that the Japanese wild populations of this species contain two genetically different groups, the Northern Population and the Southern Population. Of 21 protein loci examined four loci are nearly fixed for different alleles between these two groups, suggesting that these groups were isolated from each other in considerably ancient times. Little clinal distribution of alleles is observed at these loci, but the boundary is very distinct [1, 2].

In Japan the medaka is found in three major islands (Honshu, Shikoku, and Kyushu) and many small islands around them (Fig. 1). Thus, Aomori Prefecture seems the northernmost limit of the natural distribution of this species. The Northern Population inhabits the region along the Sea of Japan coast of northern half of Honshu. On the other hand, the Southern Population is distributed along the Pacific coast and along the western part of the Sea of Japan coast. The boundary of these two groups is well correlated with the backbone mountains of Honshu for the

most part. This species has not been found in the northern half of Iwate Prefecture (Takada, personal communication). As the northernmost limit of the Southern Population is the southern part of this prefecture, and as the medaka with the genotype of the Northern Population inhabits the Pacific coast of Aomori Prefecture, the two genetically different populations are separated by mountain barrier in the northern half of Iwate Prefecture. In this paper I report the distribution and the genetic characters of this species in the boundary region between the Northern Population and the Southern Population, mainly around the western end of the boundary where a continuous distribution of this species is observed. The object of the present study is to examine the extent of genetic isolation between the two populations and the correlation between genetic difference and geographic features in a limited area.

### MATERIALS AND METHODS

In 1983 specimens were collected in the region around the Wakasa Bay (Fig. 1); fish were then brought alive to the laboratory. Collection sites are listed below with the number of electrophoretically examined samples in parentheses:

1. Iburihashi, Kaga (12);
2. Urushibara, Sabae (10);
3. Kikuyama, Tsuruga (10);
4. Kohgasaki, Obama (10);
5. Ichiba, Maizuru (10);
6. Shimazu, Amino (10);
7. Miyajima, Toyooka (10);
8. Ohto,

Accepted May 18, 1984

Received April 17, 1984

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FIG. 1. Distribution ranges of the Japanese two major populations of *Oryzias latipes*. Closed circles show the collection sites of samples electrophoretically examined. The rectangular area is the subject of the present study, which is enlarged in Fig. 4. Populations are numbered as in the text.

Kinomoto (10); 9. Nakasuji-cho, Ayabe (11); 10. Koyama, Tottori (13).

The procedures of tissue preparation and electrophoresis were those described in Sakaizumi *et al.* [1, 2]. Electrophoretic patterns were observed for 8 enzyme systems; alcohol dehydrogenase (*Adh*), L-iditol dehydrogenase (*Idh*), superoxide

dismutase (*Sod*), phosphoglucomutase (*Pgm*), acid phosphatase (*Acp*), amylase (*Amy*), muscle lactate dehydrogenase (*Ldh*), and liver esterases. *Adh*, *Idh*, *Sod* and *Pgm* are the diagnostic enzymes of the Northern Population. *Acp*, *Amy*, and *Ldh-A* are specific markers of the Inland Sea Subpopulation and the San'in Subpopulation in the Southern

Population. As for the liver esterases, it has been known that all individuals of the Northern Population show the same pattern with two major bands, while many variants, with 1-7 bands, are found in the Southern Population [2]. Although the genetic control has not been interpreted yet, this enzyme system was used as a diagnostic marker of

the Northern Population.

## RESULTS

The allele frequencies at 7 enzyme loci are shown in Table 1. Characteristics of allelic distribution at each locus are as follows: (1) *Adh*: This locus

TABLE 1. Allele frequencies at 7 enzyme loci from 10 populations of *Oryzias latipes*

Locus	Population										
	1	2	3	4	5	6	7	8	9	10	
<i>Adh</i>	a	.08	—	.45	—	—	1.0	1.0	1.0	.95	1.0
	b	.92	1.0	.55	1.0	1.0	—	—	—	.05	—
<i>Idh</i>	a	—	—	—	—	—	—	—	1.0	1.0	.96
	b	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—	—	.04
<i>Sod</i>	a	1.0	1.0	1.0	1.0	1.0	.95	.90	—	—	—
	b	—	—	—	—	—	.05	.10	1.0	1.0	.96
	d	—	—	—	—	—	—	—	—	—	.04
<i>Pgm</i>	a	1.0	1.0	1.0	1.0	1.0	.35	—	—	—	—
	b	—	—	—	—	—	.05	—	1.0	1.0	1.0
	d	—	—	—	—	—	.60	1.0	—	—	—
<i>Acp</i>	a	1.0	1.0	1.0	1.0	1.0	1.0	.85	.20	.20	.92
	b	—	—	—	—	—	—	—	—	—	.08
	c	—	—	—	—	—	—	.15	.80	.80	—
<i>Amy</i>	a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.12
	b	—	—	—	—	—	—	—	—	—	.88
<i>Ldh-A</i>	a	—	—	—	—	.05	—	.10	—	—	—
	c	1.0	1.0	1.0	1.0	.95	.95	.90	.15	—	.08
	d	—	—	—	—	—	—	—	.85	1.0	—
	e	—	—	—	—	—	.05	—	—	—	.92

Populations are numbered as in the text.



FIG. 2. Phosphoglucumutase allozyme phenotypes from liver of three populations of medaka. Lanes 1-5, Amino; lane 6, Kaga; lane 7, Tottori.

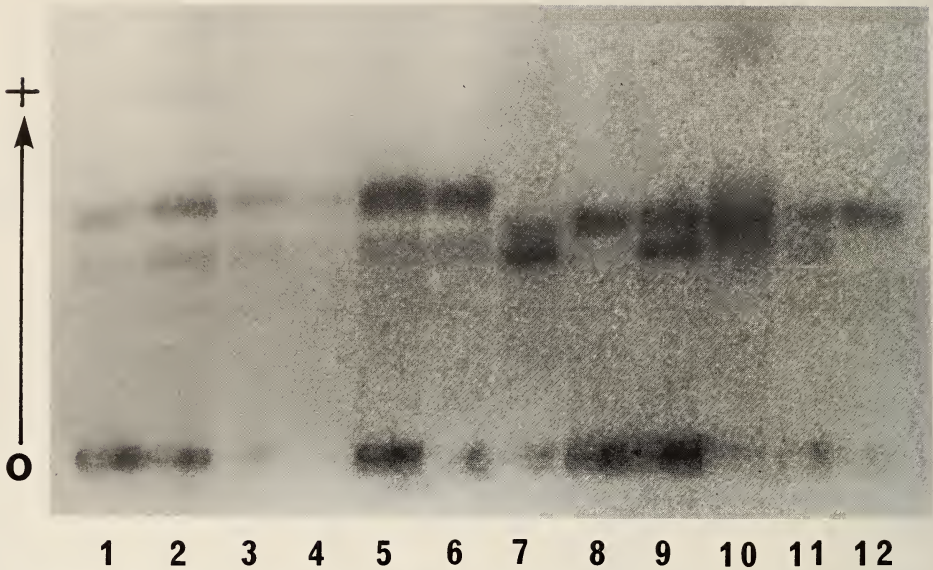


FIG. 3. Esterase allozyme phenotypes from liver of four populations of medaka. Lanes 1-5, Maizuru; lane 6, Kaga; lane 7, Tottori; lanes 8-12, Toyooka.

TABLE 2. Pattern frequency of the liver-esterases from 10 populations of *Oryzias latipes*

Pattern	Population									
	1	2	3	4	5	6	7	8	9	10
'northern'-type	1.0	.90	.60	.20	1.0	—	—	—	—	—
'southern'-type	—	.10	.40	.80	—	1.0	1.0	1.0	1.0	1.0

Populations are numbered as in the text.

was nearly fixed for *Adh<sup>a</sup>* or *Adh<sup>b</sup>*. Tsuruga population showed polymorphism. The populations from Kaga, Sabae, Obama, and Maizuru had 'northern' allele, *Adh<sup>b</sup>*. (2) *Idh* and (3) *Sod*: These loci showed similar distribution pattern of the 'northern' and the 'southern' alleles. They were essentially monomorphic in each population. The 'southern' alleles were predominant in three populations, Kinomoto, Ayabe, and Tottori. (4) *Pgm*: This locus was fixed for 'northern' allele in the populations from Kaga, Sabae, Tsuruga, Obama, and Maizuru. It was fixed for 'southern' allele in the populations from Kinomoto, Ayabe, and Tottori. An unique allele, *Pgm<sup>d</sup>*, was predominant at Amino and Toyooka (Fig. 2). (5) *Acp*: Except for three populations this locus was essentially fixed for *Acp<sup>a</sup>*. *Acp<sup>c</sup>* was frequently

observed in the populations from Kinomoto and Ayabe. (6) *Amy*: *Amy<sup>b</sup>* was found only at Tottori. This locus was fixed for *Amy<sup>a</sup>* in other populations. (7) *Ldh-A*: Kinomoto and Ayabe populations showed high frequency of *Ldh-A<sup>d</sup>*. San'in District specific *Ldh-A<sup>e</sup>* was exclusively observed at Tottori.

The 'northern' pattern of liver-esterase system was predominant at Kaga, Sabae, Tsuruga, and Maizuru (Fig. 3). The frequencies of the 'northern' and the 'southern' (=non-northern) patterns are shown in Table 2.

## DISCUSSION

Based on 8 enzyme markers, 10 populations examined in the present study can be classified

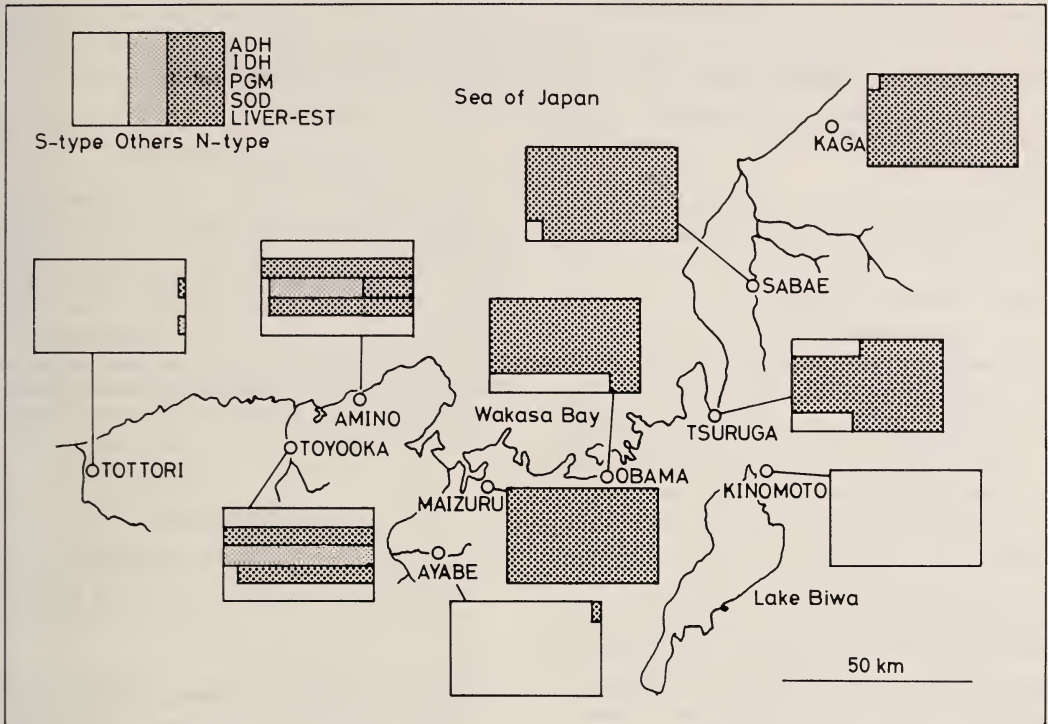


FIG. 4. Allele frequencies at four variable loci and pattern frequencies of electromorph of liver-esterases in the populations around the Wakasa Bay. N-type, 'northern'-type allele (pattern); S-type, 'southern'-type allele (patterns).

into three groups; 1) the 'northern'-type populations (Kaga, Sabae, Tsuruga, Obama, Maizuru), 2) the 'southern'-type populations (Kinomoto, Ayabe, Tottori), and 3) the boundary region populations (Amino, Toyooka). The genetic characters and distribution of these groups are illustrated in Figure 4.

The populations from Kaga, Sabae, and Maizuru show a typical genotype of the Northern Population. Although the 'southern' allele at *Adh* locus and the 'southern' patterns of liver-esterases are observed at Tsuruga and Obama, these populations can be regarded as the southernmost populations of the Northern Population. Polymorphism of *Adh* and esterases seem to be due to the secondary gene flow of the 'southern' characters into the Northern Population.

The 'southern'-type group is composed of two different populations, the Inland Sea-type (Kinomoto, Ayabe) and the San'in-type (Tottori) populations [2]. They show typical genotypes of

the Southern Population. The population from Kinomoto has quite different genotype from Tsuruga population in spite of the short geographic distance (ca. 20 km). This result suggests that the mountain barrier between these two sites has been effective to maintain the genetic isolation between these two populations. Although Ayabe and Maizuru belong to the same water system, the genotypes of populations are considerably different. This phenomenon seems to reflect the history of this drainage system (e.g. stream capture) after the establishment of the distribution of the Northern Population and the Southern Population [3].

The third group shows an unique genotype different from any of the Northern Population, the Inland Sea Subpopulation, and the San'in Subpopulation. This type of population is found in a quite limited area where is the western end of the boundary between two major populations in Japan. They have 'northern' alleles at *Idh* and *Sod* loci, while *Adh* and liver esterases are fixed

for 'southern' types. It should be emphasized that they have an unique allele, *Pgm*<sup>d</sup>. Because this allele has not been observed elsewhere, it is likely that *Pgm*<sup>d</sup> has originated and propagated recently, probably after the formation of populations in this area.

The results obtained in the present study indicate that the Northern Population and the Southern Population are isolated rigidly and that the isolation has been maintained chiefly by mountain barrier. It should be mentioned that both ends of the boundary between the two major populations are the regions where mountains sink into the sea and little flat area exists along the sea coast.

The unique genotype of the populations from the boundary region suggests that these populations were formed by an introgression between the Northern Population and the Southern Population followed by random drift at each locus. At the same time, it suggests that two major populations are not back-crossing in spite of full fertility of the laboratory reared F<sub>1</sub> hybrid of these two populations (unpublished data). In order to speculate the time when the populations of the boundary area occurred, I can find a clue that *Adh*, *Idh*, and *Pgm* loci are fixed for specific alleles, respectively, in Toyooka population. As the effective population size seems not so small, it is

possible that the origin of the boundary-region populations goes back to fairly old times, for instance, to the last glacial period or so.

#### ACKNOWLEDGMENTS

I am grateful to Professor Nobuo Egami of the University of Tokyo for his useful commentary on the early draft of the manuscript. Kind help in the collection of materials by Kiyoshi Naruse is greatly appreciated. I thank Mr. Muneo Takada of Iwate Education Center for Sciences for allowing me to use his unpublished data. This work was supported in part by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, Science and Culture to Professor N. Egami.

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